18TH ANNUAL SURGERY RESEARCH CONFERENCE

DEPARTMENT OF SURGERY
THE OHIO STATE UNIVERSITY

18TH ANNUAL SURGERY RESEARCH CONFERENCE

BIOMEDICAL RESEARCH TOWER, ROOM 115
THURSDAY, MAY 23, 2013
Welcome

Welcome to the 18th Annual Department of Surgery Research Conference! This conference is designed to bring students, residents, fellows, faculty and guests together to share and discuss results of research relevant to a variety of surgical disciplines. It is also an opportunity for Department of Surgery (DOS) residents, graduate students and postdoctoral research trainees to develop their scientific communication skills. Each year the Department of Surgery, invites a leader in Surgery to visit The Ohio State University and get to know the students and faculty in the department through a variety of activities including participation as a faculty judge at the Annual DOS Research Conference. This year we are delighted to have Dr. Carlos A. Pellegrini, the Henry N. Harkins Professor and Chair, Department of Surgery, University of Washington, as our guest. In addition to the visiting professor, we also ask a prominent research leader at Ohio State to participate in our conference by serving on a panel of faculty judges. Dr. Rebecca Jackson, Associate Dean for Clinical Research and Professor of Internal Medicine, has generously agreed to participate.

Over the years the format for the conference has developed into two oral sessions separated by a poster session. The oral and poster presentations are competitively selected based on the quality of the science, impact of the work, and novelty and diversity of the topic. DOS faculty serve as “Faculty Discussants” and comment on the presentation to put the work into context for the audience and stimulate additional discussion. We invite medical students on their 3rd year Surgery Clerkship to become active participants by reviewing the topic relevant to the research abstract and preparing questions or comments for the presenter. Many of the residents who participate in this conference are trainees in the Department of Surgery Master of Medical Science Program which includes structured didactics in Research Design, Biostatistics, Research Ethics, Scientific Communication (including grant writing) and Electives relevant to the area of research.

Ginny Bumgardner, MD, PhD
Associate Dean for Research Education
Agenda
Thursday, May 23, 2013

Welcome and Introduction of Visiting Professor
Welcome and introduction by E. Christopher Ellison, MD, the Robert M. Zollinger Endowed Chair and College of Medicine Distinguished Professor, CEO of the Faculty Group Practice, Senior Associate Vice President for Health Sciences, Vice Dean of Clinical Affairs, The Ohio State University College of Medicine

Introduction to the Conference
Conference purpose and format by Ginny L. Bumgardner, MD, PhD, Professor of Surgery, Division of Transplantation, Associate Dean for Research Education, Director, Master of Medical Science Program, The Ohio State University College of Medicine

Judges
Carlos A. Pellegrini, MD, FACS, FRCSI (Hon), E. Christopher Ellison, MD, and Rebecca D. Jackson, MD

Moderator
Session 1 and 2 moderated by Ginny Bumgardner, MD, PhD

Session 1: Oral Presentations, 7:45 to 9:00 a.m.
Wound-site macrophages from chronic wound patients regulates keratocyte signaling. Kasturi G. Barki, MD • Faculty Advisor: Sashwati Roy, PhD • Discussant: William Carson, MD .................................................................11

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Wound macrophage dysfunction in diabetes: significance of MFG-E8. Amitava Das, M Pharm • Faculty Advisor: Sashwati Roy, PhD • Discussant: Michael Go, MD, MS .................................................................15

A pilot study of interferon-alpha-2b dose reduction in melanoma shows that high doses are not necessary for optimal immune activation. Sara Martin del Campo, MD • Faculty Advisor: William Carson, MD • Discussant: Kyle Perry, MD .................................................................16
Break and Poster Presentations, 9:15 to 10:00 a.m.

Does delay in appendectomy affect surgical site infection in children with appendicitis? Laura Boomer, MD • Faculty Advisor: Gail Besner, MD

Multivariate analysis of risk factors for surgical site infection after laparoscopic colorectal surgery. Joseph Drosdeck, MD • Faculty Advisor: Syed Husain, MD

Latent murine cytomegalovirus changes the composition and function of immune cells in the lung. Varun Dwivedi, PhD • Faculty Advisor: Charles Cook, MD

Sonographic evaluation of intravascular volume status in the surgical intensive care unit: a prospective comparison of subclavian vein and inferior vena cava collapsibility index. Alistair Kent, MD, MPH • Faculty Advisor: Stanislaw Stawicki, MD

HB-EGF augments the ability of mesenchymal stem cells to attenuate intestinal injury. Daniel Watkins, MD • Faculty Advisor: Gail Besner, MD

Hyponatremia increases expression of apoptosis-related proteins and infarct size. Weiping Ye, PhD • Faculty Advisor: Juan Crestanello, MD

Session 2: Oral Presentations, 10:00 a.m. to 12:00 p.m.

Adaptations in gene and protein expressions within different regions of the left ventricle after an acute myocardial infarction. Tyler Spata, MD • Faculty Advisor: Ahmet Kilic, MD • Discussant: Mounir Haurani, MD

Methylene blue infusion for treatment of traumatic brain injury-associated neuroinflammation and depressive complications. Ashley Fenn, BS • Faculty Advisor: Daniel Eiferman, MD and Jonathan Godbout, PhD • Discussant: Sashwati Roy, PhD

MicroRNA-1 induction impairs ischemic wound healing by attenuating keratinocyte migration and angiogenesis. Jaideep Banerjee, MS • Faculty Advisor: Chandan Sen, PhD • Discussant: Charles Cook, MD

Near infrared fluorescent cholangiography (NIRF-C) for biliary imaging during laparoscopic cholecystectomy. Mark Wendling, MD, MS • Faculty Advisor: W. Scott Melvin, MD • Discussant: Mark Bloomston, MD

The role of microRNA-155 in a murine model of non-alcoholic steatohepatitis. Rachael Sullivan, MD • Faculty Advisor: Samson Jacob, PhD • Discussant: Sylvester Black, MD, PhD

Keratinocyte-directed conditional ablation of dicer impairs would healing via induction of P21^{WAF1/CIP1}. Subhadip Ghatak, PhD • Faculty Advisor: Chandan Sen, PhD • Discussant: John Phay, MD
Visiting Professor

Carlos A. Pellegrini, MD, FACS, FRCSI (Hon)

Dr. Pellegrini is the Henry N. Harkins Professor and Chair of the Department of Surgery at the University of Washington. He is a world-renowned surgeon in the area of esophageal diseases and introduced minimally invasive operations for the treatment of achalasia and gastroesophageal reflux.

Dr. Pellegrini was born to two physicians, and spent his childhood years in rural Argentina. He attended the University of Rosario Medical School, graduating in 1971 and remained there for surgical training until 1975. He emigrated to the U.S. in 1975 and completed his surgical residency at the University of Chicago. He accepted a faculty appointment at the University of California, San Francisco in 1979. In 1993 he assumed the chairmanship of the Department of Surgery at the University of Washington.

As a member of the Accreditation Council for Graduate Medical Education (ACGME), Dr Pellegrini was involved in the process of limiting U.S. medical residents to an 80-hour work week. He was a member of the American Surgical Association’s Blue Ribbon Committee, which delivered an influential report on this topic and on surgical education in 2005. He has pursued research into efficient and safe methods of handing-off patients between shifts of residents. During his tenure, the Department of Surgery has established the Institute for Simulation and Interprofessional Studies (ISIS), a center for education and training through various forms of simulation.

Dr. Pellegrini was named to the Robert Wood Johnson Foundation Clinical Scholars Program’s National Advisory Committee in November 2008. The committee is responsible for overseeing the foundation’s Clinical Scholars Program and selecting its participants. He is the former president of the American Surgical Association and is currently the president-elect of the American College of Surgeons.

His clinical interests focus in the area of benign and malignant esophageal problems, minimally invasive surgery, and surgery of the esophagus, stomach and gall bladder.

As a researcher, Dr. Pellegrini has investigated a wide variety of disorders and surgical procedures. He has been involved in surgical education research and has been involved in the University of Washington’s Mini-Medical School, an annual series of lectures and seminars on medical topics open to the public. He devotes his time to training individuals to become “total” doctors, not just surgeons.
Rebecca D. Jackson, M.D.

Rebecca D. Jackson, M.D., began as a basic scientist, and over time, she grew interested in medically related questions. She was awarded her BS degree in 1975 and medical degree in 1978, both from The Ohio State University. Following a residency in internal medicine at Johns Hopkins University, (1978-1981), she returned to Ohio State for a fellowship in endocrinology and a residency in physical medicine and rehabilitation, (1981-1983). She is currently Associate Dean for Clinical Research in the College of Medicine, Director of the OSU Center for Clinical and Translational Science and Professor of Internal Medicine, Division of Endocrinology, Diabetes and Metabolism

In the early 1990s, Dr. Jackson earned a spot as one of the research leaders of the Women’s Health Initiative (WHI). This important project followed more than 161,000 postmenopausal American women from diverse ethnic and geographic backgrounds for several years, beginning in 1993. WHI scientists have been studying many conditions beyond osteoporosis and bone fractures, including cardiovascular disease, breast and colorectal cancer, and dementia. Collectively, these are among the most common causes of death, disability, and poor quality-of-life among postmenopausal women.

Dr. Jackson is a national leader on studies of osteoporosis, and its causes and therapies. She has studied the effects of estrogen, progesterone, calcium, hormonal therapy and pharmaceutical agents on bone metabolism. Her work has also been directed into related areas, including diagnosis, radiological techniques, the role of ethnicity and of mechanical approaches to preserve muscle strength and bone structure. She is published in leading clinical journals including the New England Journal of Medicine, the Journal of the American Medical Association, and the Annals of Internal Medicine.

Dr. Jackson’s awards included: a fellowship from the Kellogg Foundation (1982-1985) for leadership, Clinical Associate Physician from the NIH (1984-1987), Physician Scientist Award from NIH (1987-1992), and elected Fellow of the American Association for the Advancement of Science (2008). Her awards as a woman or on behalf of women include: Woman of the Year by Beta Sigma Phi in 1997, Ohio Women’s Hall of Fame (1988), Disabled Professional Woman of the Year from the Pilot Club (1988), Local Legend by the American Medical Women’s Association and the National Library of Medicine (2005), and an award for research from Women for Economic and Leadership Development (2008). She has chaired committees of the Woman’s Health Initiative at several levels, including nationally in Washington, DC. Dr. Jackson is also the Director of the OSU Center for Women’s Health, and the principle investigator for the NIH Clinical and Translational Awards grant.
Presenters

Jaideep Banerjee, MS
Graduate Research Associate
**Hometown:** Calcutta, India  
**BS:** Physiology, University of Calcutta, India  
**MS:** Molecular Biology, University of Calcutta, India  
**Research interests:** MicroRNAs in tissue injury and repair

Kasturi Barki, MD
Post Doctoral Researcher
**Hometown:** Bangalore, India  
**MD:** Mahadevappa Rampure Medical College, Gulbarga University, Karnataka State-INDIA  
**Research interests:** Inflammation and wound healing

Laura Boomer, MD
Research Fellow
**Hometown:** Defiance, OH  
**BA:** Psychology, Marquette University, Milwaukee, WI  
**MD:** Medical College of Ohio, Toledo, OH  
**Additional training:** Master of Physical Therapy, Marquette University  
**Research interests:** Clinical outcomes, tissue engineering

Amitava Das, M. Pharm
Graduate Research Associate
**Hometown:** Kolkata, India  
**B. Pharmacy:** PES College of Pharmacy, Bangalore, India  
**Additional training:** M. Pharmacy, PES College of Pharmacy, Bangalore, India  
**Research interest:** Wound healing, Inflammation, MicroRNA,

Joseph Drosdeck, MD
General Surgery Resident
**Hometown:** Milwaukee, WI  
**BA:** Psychology, University of Wisconsin - Milwaukee, WI  
**MD:** University of Wisconsin, Madison, WI  
**Research Interests:** Minimally invasive surgery, surgical education
Presenters

Varun Dwivedi, PhD
Post-Doctoral Researcher
Hometown: Aligarh, India
BS: Biology, B.R. Ambedkar University, Agra, India
PhD: Biochemistry, Aligarh Muslim University, Aligarh, India
Research interest: Viral immunology and pathogenesis

Ashley Fenn, BS
Graduate Research Associate
Hometown: Centennial, CO
BS: Biochemistry and Zoology, Colorado State University, Fort Collins, CO
Research interest: Influence of aging and traumatic brain injury on behavior and microglia function

Subhadip Ghatak, PhD
Post-Doctoral Researcher
Hometown: Kolkata, India
BS: Physiology, University of Calcutta, Calcutta, India
MS: Physiology, University of Calcutta, Calcutta, India
PhD: West Bengal University of Health Science, Kolkata, India
Research interest: MicroRNA in tissue injury and repair

Jon Henry, MD, MS
General Surgery Resident
Hometown: Geneva, Ohio
BS: Biology, Ohio State University, Columbus, OH
MS: The Ohio State University
MD: The Ohio State University

Alistair Kent, MD, MPH
General Surgery Resident
Hometown: Walnut Ridge, AK
BS: Mathematics and Chemistry, Harding University, Searcy, AK
MD: Washington University in St. Louis, St. Louis, MO
MPH: George Washington University, Washington, DC
Research Interest: National health program, health workforce, critical care policy
Presenters

Sara Martin del Campo, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Peoria, IL
BS: Biology, Indiana University, Bloomington, IN
MD: The University of Iowa Carver College of Medicine, Iowa City, IA
Research interest: microRNA expression in melanoma

Mika Matthews, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Battle Creek, MI
BS: University of Michigan, Ann Arbor, MI
MD: Morehouse School of Medicine, Atlanta, GA
Research Interest: Intestinal inflammation and repair

Robert Plews, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: New Port Richey, FL
BS: Microbiology, University of South Florida, Tampa, FL
MD: University of South Florida College of Medicine, Tampa, FL
Research interest: Thyroid cancer with particular focus on tumor biology and metabolism

Tyler Spata, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Spring, TX
MD: The University of Texas Medical Branch, Galveston, TX
Additional training: Cardiovascular Research, The University of Texas Health Science Center, Houston, TX; Cardiovascular Surgery/Anesthesia Research, Texas Heart Institute, Houston, TX
Research interest: Cardiac ischemia, cardiac transplantation, mechanical cardiac-assist devices, and revascularization techniques.

Rachael Sullivan, MD
General Surgery Resident, Master of Medical Science Program Candidate
Hometown: Delta, OH
BA: Biology, University of Toledo, Toledo, OH
MD: The Ohio State University, Columbus, OH
Research interest: Hepatocellular carcinoma
Presenters

Daniel Watkins, MD
Pediatric Surgery Research Fellow
Hometown: Kalamazoo, MI
BA: Chemical Engineering, University of Michigan, Ann Arbor, MI
MD: The Ohio State University, Columbus, OH
Research Interest: Mesenchymal stem cells, intestinal injury

Mark Wendling, MD, MS
General Surgery Resident
Hometown: Mayville, WI
BA: Biology, Lawrence University, Appleton, WI
MS: Medical Science, The Ohio State University
MD: Medical College of Wisconsin, Milwaukee, WI
Research interest: Minimally invasive surgery

Weiping Ye, PhD
Research Specialist
Hometown: Longyou, Zhejiang, China
MD: Tianjin College of Medicine, Tianjin, China
MPH: Chinese Academy of Preventive Medicine, Beijing, China
PhD: The Ohio State University College of Veterinary Medicine, Columbus, OH
Research interest: Mechanisms of higher mortality in cardiac patients with hyponatremia

Special thanks to the following Med III Students for volunteering as student discussants:

John Birchak  Richard Price
Patrick Grierson  Trevor Quinn
Elizabeth Herman  Casey Reed
Alexander Kuley  Nathan Schulz
Michael Morgan  Nikhil Sebastian
Joel Palko
Wound-site macrophages from chronic wound patients regulates keratonooye signaling

Kasturi Ganesh Barki, MD, Amitava Das, MS, Savita Khanna, PhD, Piya Das, MS, Urmila Gnyawali, RN, Gayle M. Gordillo, MD, Chandan K. Sen, PhD, Sashwati Roy, PhD

Introduction: We have successfully isolated functional macrophages from the wound site of chronic wound patients. A transcriptome screening study was performed comparing wound site macrophages with matching blood derived macrophages from the same chronic wound patient. Higher oncostatin M (OSM) expression in wound site macrophages represents a foundation observation on which this study rests. High levels of OSM (p<0.01; n=19) were observed in chronic wound fluids as compared to plasma from the same subjects. OSM is a potent activator of keratinocytes. We hypothesized that OSM produced by wound macrophages interacts with keratinocytes at the wound site to drive cell signaling towards healing

Methods: Adult chronic wound patients (25-80 years old) undergoing VAC® (negative pressure) therapies (NPWT) of their wounds at the Comprehensive Wound Center (CWC) were recruited. Wound fluid was derived from the NPWT dressing by lavaging the wound dressing with saline solution. In addition, paired peripheral blood samples were collected from each patient. Blood monocytes from corresponding subjects with chronic wounds were isolated using a Ficoll-Hypaque density gradient. Human keratinocyte HaCaT were treated either with wound fluid or recombinant OSM.

Results: Treatment of human keratinocytes with OSM resulted in dose dependent induction of miRNA-203. miR-203 is abundantly expressed in the skin and is involved with the differentiation of keratinocytes. OSM down regulated keratinocyte SOCS3 protein expression. Elevated SOCS3 impairs epithelial repair of cutaneous wounds by blocking keratinocyte proliferation and migration. The role of SOCS3 in chronic wounds is therefore of outstanding interest. AntagomiR and miR mimic experiments demonstrated that miR-203 directly silences SOCS3.

Conclusions: SOCS3 is a key regulatory molecule that inhibits JAK-Stat signaling pathway in keratinocytes. SOCS3 is overexpressed in the wound margin epithelia of diabetic wounds. This work provides first evidence that OSM, produced by human wound-site macrophages, induces miR-203 expression in human keratinocytes silencing SOCS3 and supporting healing.
A novel AMPK activator inhibits thyroid cancer cell growth

Robert L. Plews, MD, Adlina Mohd Yusof, PHD, Chaojie Wang, BS,- Motoyasu Saji, MD, Ching-Shih Chen, PHD, Matthew D. Ringel, MD, John E. Phay, MD

Introduction: The role of 5’ adenosine monophosphate-activated protein kinase (AMPK) as a modulator of energy utilization, in response to intracellular energy availability, is well established. Recent studies have demonstrated its role in tumor biogenesis and metabolism, thus making it a promising target for anti-cancer therapy. OSU-53 is a novel thiazolidinedione-derived direct AMPK activator, which has been shown to have in vitro and in vivo anti-tumor activity against triple negative breast cancer cell lines and their xenografts in nude mice. In this study, the effects of OSU-53 on growth and proliferation of several human thyroid carcinoma cell lines were determined, as well as its influence on key oncogenic signaling pathways.

Methods: Cell lines utilized consisted of undifferentiated (SW1736, Hth104, Hth7, C643) and differentiated (BCPAP, FTC-133) human thyroid carcinoma cells. Cell growth assays were performed after treatment with various concentrations of OSU-53. To characterize the influence of OSU-53 on oncogenic signaling pathways, cells were treated with 5µM OSU-53 and collected at various time points. Immunoblotting for AMPK$\alpha_{1,2}$/p-AMPK$\alpha_{1,2}$, p70S6k/p-P70S6k, TSC/p-TSC, S6/p-S6 and GAPDH was performed.

Results: Cell growth was effectively inhibited at doses as low as 5µM, and as early as 48 hours of drug treatment in all cell lines. Undifferentiated cell lines appeared to be more sensitive to drug treatment than differentiated cell lines. Immunoblot analysis revealed a time-dependent activation of AMPK, as well as downstream inhibition of the Raptor/mTOR pathway, as evident by down-regulation of p-P70S6k and p-S6 which are phosphorylated as a result of mTOR activation. There is also activation of TSC, which has been shown to be regulated by AMPK activation.

Conclusion: OSU-53 effectively inhibited tumor cell growth and proliferation in a time and dose-dependent manner, with undifferentiated cell lines being the most sensitive. The primary mechanism of action appears to be in part, down regulation of the Raptor/mTOR pathway through activation of AMPK and TSC. These findings indicate the potential of OSU-53 in the development of thyroid cancer treatment.
MicroRNA from cyst fluid differentiates cystic lesions of the pancreas

Jon C. Henry MD, Jinmai Jiang PhD, Claudio Bassi MD, Giovinazzo Francesco MD, Thomas Schmittgen PhD, Mark Bloomston MD

Introduction: Prognostication for cystic neoplasms of the pancreas continues to evolve. Beyond simple size and CEA determination, microRNA (miRNA) promises the potential for a molecular signature for cancer risk. In this study we sought to identify miRNAs that could predict malignant potential of pancreatic cystic lesions.

Methods: RNA was harvested from the cyst fluid of 72 patients with cystic neoplasms of the pancreas. Samples with adequate RNA (≥10 pg/nL) were then selected to undergo profiling by real time PCR of the 379 most common human miRNAs. All patients underwent resection and miRNA profiles were correlated with histopathology grouped by benign (serous cystadenomas), premalignant (intraductal papillary mucinous neoplasms and mucinous cystadenomas), and malignant lesions (adenocarcinoma).

Results: Adequate RNA for analysis was obtained from 42 (58.3%) of the samples. Malignant lesions were more likely to have adequate RNA (N=17, 81%) than either benign (n=6, 33%) or pre-malignant lesions (n=19, 59%) (p = 0.011). Nine miRNA were identified as having a significant differential expression between benign and premalignant or malignant lesions. As the number of miRNA expressed by each sample increased beyond the median for the entire set the more likely the sample was to be premalignant or malignant (Figure 1). All premalignant or malignant lesions expressed at least one miRNA beyond the median whereas no benign lesions express less than four and only two expressed more than zero miRNA above the threshold.

Conclusions: The presence of RNA in cyst fluid from patients with pancreatic cystic neoplasms may, in itself, be a predictor of premalignancy or, more likely, malignancy. miRNA can be utilized to further differentiate between purely benign, premalignant, and malignant cystic lesions of the pancreas.
The protective effects of HB-EGF in a rat model of cerebral palsy: a pilot study

Mika A.B. Matthews, MD, Palak Painter, MS, William E. Carson, MD, Gail E. Besner, MD

Introduction: Cerebral Palsy (CP) consists of a collection of movement and posture disorders caused by non-progressive injuries during fetal and infant brain development. Individuals affected by CP have mental retardation, epilepsy, abnormal behavior, perception disorders, or language dysfunction. Brain damage following inflammation due to ischemia, known as hypoxic-ischemic encephalopathy (HIE), is a major cause of CP. Previous studies have shown that heparin-binding EGF-like growth factor (HB-EGF) protects multiple organs including the brain from injury by decreasing reactive oxygen species production and inflammatory cytokine production. In addition, HB-EGF stimulates neurogenesis. The objective of the current study was to evaluate the effect of HB-EGF in an animal model of cerebral palsy.

Methods: Seven day old Sprague Dawley rat pups were randomized into 4 groups: 1) non-operative (n=2), 2) sham injury (n=2), 3) HIE injury (n=1), and 4) HIE injury + HB-EGF treatment (n=2). Rats in the HIE injury group underwent permanent left common carotid artery ligation followed by 2 hours of exposure to 8% O₂ and 92% N₂ in a hypoxic chamber. Pups in the sham injury group underwent exploration of the neck without arterial ligation, followed by 2 hours in a normoxic chamber containing room air. At 2 and 22 hours after injury, HB-EGF-treated pups received intranasal drops of HB-EGF (1ìg in 20ìL of saline) or 20ìL of saline. Two days after injury, animals underwent intracardiac perfusion with neutral buffered formalin, and sections of the brain containing the hippocampus (memory) and internal capsule (motor execution) were harvested. Histologic sections were stained with anti-glial fibrillary acidic protein (GFAP) antibodies for identification of astrocytes, anti-myelin basic protein (MBP) antibodies for identification of myelin sheaths covering neuronal axons, and DAPI for identification of nuclei. Quantification of GFAP, MBP, and DAPI immunohistochemical staining was performed using image J software and averaged per high-powered field (HPF).

Results: Pups exposed to HIE had decreased MBP staining in the internal capsule compared to pups exposed to sham surgery or no surgery (45 counts/HPF vs. 133 and 100.5 counts/HPF, respectively). HIE-injured animals treated with HB-EGF had increased MBP staining compared to non-HB-EGF treated HIE-injured animals (153.5 counts/HPF vs. 45 counts/HPF). HIE also led to a decrease in GFAP-positive astrocytes in the hippocampus compared to sham and no surgery (64 cells/HPF vs. 90 and 90 cells/HPF, respectively). HB-EGF treatment resulted in an increase in GFAP-positive astrocytes compared to non-HB-EGF-treated HIE-injured animals (227 cells/HPF vs. 64 cells/HPF).

Conclusions: The results of this pilot study suggest that administration of HB-EGF preserves myelination of axons in the internal capsule and increases the number of astrocytes in the hippocampus in a model of cerebral palsy. Ongoing studies will confirm these preliminary findings, and will determine whether these findings translate into preservation of motor and memory function in animals with cerebral palsy.
Wound macrophage dysfunction in diabetes: significance of MFG-E8

Amitava Das, Savita Khanna, Chandan K. Sen and Sashwati Roy.

Introduction: Impaired cutaneous wound healing is a debilitating complication encountered during diabetes mellitus. Efferocytosis in wound macrophages from diabetic mice was markedly impaired than non-diabetic animals. We hypothesized that MFG-E8 which facilitates macrophage dead cell clearance activity, gets glycated in diabetes, thereby dampening dead cell recognition by wound macrophages and eventually leading to impaired cutaneous wound healing.

Methods: To address the hypotheses we utilized mice lacking MFG-E8 protein which were subjected to excisional cutaneous wounding and sponge implantation for wound macrophage harvesting.

Results: Cutaneous wound healing was significantly (p<0.05; n=5) impaired in MFG-E8 null mice compared to matching wild-type suggesting a crucial role of this protein in wound repair process. Wound macrophages isolated from MFG-E8-/- mice were impaired in their ability to clear dead cell and produced high levels of pro-inflammatory cytokines (TNF-α). The presence of arginine residues on MFG-E8 renders this protein susceptible to glycation. Using ELISA and Biacore assays we demonstrate that glyoxal, a product of glucose oxidation, can glycate MFG-E8. Once glycated, the affinity of MFG-E8 for binding with PS was markedly diminished.

Conclusion: Glycation of MFG-E8 in diabetic macrophages represent one of the major mechanisms that impair dead cell recognition and clearance by wound macrophages in diabetic wounds. Such dysfunction in macrophage activity is in direct conflict with the resolution of inflammation resulting in chronic inflammation noted in diabetic wounds. So it can be concluded that MFG-E8 gets glycated in diabetes mellitus leading to dampened dead cell recognition by wound macrophages thus causing impairment in cutaneous wound healing.
A pilot study of interferon-alpha-2b dose reduction in melanoma shows that high doses are not necessary for optimal immune activation.


Introduction: High dose interferon-alpha-2b (IFN-α) is currently the only FDA-approved adjuvant therapy for patients who have undergone surgery for high-risk melanoma lesions (primary tumors > 4mm or lymph node metastases). Standard therapy includes 20 treatments of intravenous IFN-α at 20 million units (MU)/m² over four weeks followed by subcutaneous (s.c.) dosing at 10 MU/m² three times weekly for an additional 11 months. However, this high dose regimen calls for prolonged periods of therapy during which nearly 50% of patients require treatment delays or dose reductions due to constitutional, hepatic, or neurologic symptoms. We have shown that IFN-α-induced activation of the Janus kinase-signal transducer and activator of transcription (Jak-STAT) pathway within the host immune system is responsible for the anti-tumor effects of this cytokine (Lesinski, et.al, JCI, 2003). By examining Jak-STAT activation via flow cytometry in patient peripheral blood mononuclear cells (PBMCs), we can use signal transduction within immune effector cells as a surrogate marker of IFN-α action in patients undergoing immunotherapy. We hypothesized that, compared to high dose IFN-α, lower doses of IFN-α would be better tolerated and equally effective in activating Jak-STAT signal transduction.

Methods: We conducted an IRB-approved, investigator-initiated study, in which patients eligible for adjuvant IFN-α initially received standard dose IFN-α followed by steady dose reductions. Patients enrolled were considered candidates for adjuvant IFN-α therapy after having undergone successful surgery for high risk melanoma. The patients received the standard 20 treatments of intravenous IFN-α at 20 MU/m² over four weeks and then began the standard dose of s.c. IFN-α at 10 MU/m² three times weekly. After four weeks at 10 MU/m², the dose of IFN-α was reduced to 8 MU/m² for four weeks and then to 6 and 4 MU/m² at four week intervals. Patients continued on the lowered s.c. dose for a total of 11 months. Peripheral blood was drawn pre-therapy and at two week intervals just prior to the treatment administration and again at one hour and four hours post-treatment. PBMCs were isolated from peripheral blood via Ficoll-Paque density centrifugation and evaluated for levels of non-phosphorylated STAT1 and STAT2 and activated (phosphorylated) STAT1 (pSTAT1) and STAT2 (pSTAT2) by intracellular flow cytometry (Lesinski, et.al, JNCI, 2004). Specific fluorescence of pSTAT1 and pSTAT2 was then compared at the various doses by the Wilcoxon signed rank test.

Results: Thirty-four patients participated in the study. The average age was 52 years (range 22-77) and there were 18 males and 16 females. There was one Hispanic patient and 33 non-Hispanic patients. Twenty-four primary tumors (71%) were Clark’s level IV. Superficial spreading was the most common histologic subtype. Twenty-six patients (76%) had lymph node involvement, and 16 (47%) had ulceration of the primary. Fifteen patients (44%) completed the entire year of therapy. The toxicities encountered were consistent with those normally seen with IFN-α administration. There was only one grade 4 toxicity, which consisted of transient lymphopenia. There were 81 grade 3 toxicities including leukopenia, fatigue and hypophosphatemia. Most of these occurred at the higher IFN-α doses, and only five patients (15%) had their treatment stopped as a result of an IFN-related toxicity. This compares favorably to other studies of adjuvant IFN-α in which 23% of patients had treatment stopped due to an IFN-related toxicity. The mean level of pSTAT1 at dose level 10 MU/m² was 5.96 (0.31 19.1), and the mean level of pSTAT1 at dose level 4 MU/m² was 4.80 (0.11–21.17). This difference was not statistically different (p = 0.22). Similar results were obtained for levels of pSTAT2. Age, gender, mitotic rate, and history of lymph node involvement had no influence on this metric. The overall survival (OS) from the time of surgery at 100 months was 60%. The median time to recurrence after surgery was 61.3 months.

Conclusions: Dose reduction of IFN-α does not lead to diminished Jak-STAT signal transduction within immune cells and may be better tolerated by patients.
Does delay in appendectomy affect surgical site infection in children with appendicitis?

Laura A. Boomer, Jennifer Cooper, Katherine J. Deans, Peter C. Minneci, Karen Leonhart, Brian D. Kenney, Karen Diefenbach, Gail E. Besner

Introduction: It has been suggested that delay in appendectomy in patients with acute appendicitis does not lead to increased surgical site infections (SSI). However, some recent adult literature challenges this. Our goal was to investigate the association of time to surgery with SSI in children undergoing appendectomy.

Methods: Patients undergoing appendectomy for appendicitis from 1/1/10-12/31/12 were included for study. We collected data on patient demographics including obesity and other risk factors, length of symptoms, time of initial emergency department (ED) presentation, time of admission to the surgical service, time at start of operation, antibiotic administration, and occurrence of SSI (wound infection or abdominal/pelvic abscess). Times from ED triage to operation were grouped into <4h, 4-8h, 8-12h, 12-16h, and >16h for analysis. Times from admission to the surgical service to operation were grouped into <3h, 3-6h, 6-9h, 9-12h, and >12h. Simple appendicitis (SA, acute) was differentiated from complex appendicitis (CA, gangrenous/ruptured). Interval appendectomy patients were excluded from analysis. Patients undergoing delayed appendectomy were treated with intravenous antibiotics until surgery. Chi-square and Fisher’s exact tests were used to compare comorbidities and time variables between patients with SA vs. CA. Cochran-Armitage tests for trend were used to evaluate differences in SSI rates across increasing categories of each time variable. Univariable and multivariable logistic regression models were used to evaluate associations between time to appendectomy and the development of SSI in the overall cohort and in SA and CA subgroups.

Results: Patients with SA (n=919) or CA (n=469) were analyzed. SSI occurred in 1.4% of SA and 12.4% of CA patients ($p<0.0001$). Patients with CA were more likely to have an open operation or laparoscopic-to-open conversion than those with SA (15.4% vs. 5.9%, $p<0.0001$). In univariate analysis, obesity (Odds Ratio 2.6, $p=0.04$) and increased admission WBC count (Odds Ratio 1.06, $p=0.01$) were each associated with increased SSI. The presence of obesity or other risk factors did not differ between patients with SA and CA. The risk of SSI did not significantly increase as the length of time between ED triage and operation increased (ALL patients, $p=0.51$; SA patients, $p=0.91$; CA patients, $p=0.44$). Likewise, the risk of SSI did not significantly increase as the duration of time from surgical admission to operation increased (ALL patients, $p=0.997$; SA patients, $p=0.69$; CA patients, $p=0.96$). Greater length of symptoms was associated with increased SSI ($p<0.05$ for ALL, SA and CA patients). In multivariable models, only CA was a significant risk factor for SSI ($p<0.0001$).

Conclusion: We found no significant increase in SSI related to delay in appendectomy in patients treated with intravenous antibiotics until surgery. A future multi-institutional study is planned to confirm these single-institution results.
Multivariate analysis of risk factors for surgical site infection after laparoscopic colorectal surgery

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Introduction: Surgical site infection (SSI) and incisional hernia (IH) are among the most common complications after colorectal surgery. While many risk factors for these complications are unavoidable, evidence suggests that use of Pfannenstiel incisions for specimen extraction during laparoscopic procedures may reduce their incidence. The objectives of this study were to identify risk factors for extraction site SSI (primary objective) and IH (secondary objective) in patients undergoing laparoscopic colorectal surgery.

Methods: Patients who underwent laparoscopic colorectal resections at The Ohio State University Wexner Medical Center between January 2006 and October 2012 were included. In addition to reviewing of medical records, data was gathered from patient questionnaires with a focus on two endpoints: extraction site SSI and IH. Univariate logistic regression analysis was performed to identify significant associations between the two endpoints and the following variables: age, gender, ASA (American Society of Anesthesiologists’) score, cancer, inflammatory bowel disease (IBD), body mass index (BMI), diabetes, chronic obstructive pulmonary disease (COPD), use of immunosuppressant medications, chemotherapy, radiation therapy, smoking, surgical history, surgery duration, duration of follow-up, use of hand-assistance, and utilization of Pfannenstiel incisions for specimen extraction. Multivariate analysis was performed for significant variables.

Results: A total of 419 patients met inclusion criteria. The incidence of SSI was 10.3%. Higher BMI, presence of IBD, younger age and hand-assisted procedures were associated with a significantly higher risk of SSI. Use of Pfannenstiel extraction sites was associated with lower infection rates; however this association did not reach statistical significance. IBD, BMI and hand-assistance retained statistical significance with multivariate analysis. Odds ratios for SSI with IBD, hand-assistance and BMI (per unit increase) were 3.3, 2.2, and 1.06, respectively.

Conclusions: Alterations in surgical technique and specimen extraction site can reduce wound-related complications after laparoscopic colorectal resections. Remaining risk factors are largely non-modifiable from a surgeon’s perspective.
Latent murine cytomegalovirus changes the composition and function of immune cells in the lung

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Introduction: Human cytomegalovirus (HCMV) is a ubiquitous virus infecting 40-80% of the human population. After primary infection, HCMV persists for life despite a vigorous antiviral response. Reactivation of latent HCMV can lead to serious outcomes in immunosuppressed and immunocompetent hosts experiencing critical trauma and stress. Lungs are a major target organ for cytomegalovirus latency and recurrence. CMV reactivation occurs in lungs of ~33% of immune competent patients during critical illness. Our hypothesis is that latent CMV infection alters immune function in lungs in a manner that predisposes immune competent hosts to severe lung injury upon trauma. To test this hypothesis, we developed a mouse model of CMV-related lung injury to investigate the immunological environment in lungs of latently-infected mice.

Methods: BALB/c mice were inoculated with PBS or with 1X10^6 PFU of the Smith strain MCMV by the intraperitoneal route. After viral latency was well established (6 months after primary infection), lung mononuclear cells were isolated from the perfused lungs of each group of mice, reacted with antibodies to lymphocyte, myeloid and innate immune cell specific markers, and analyzed by flow cytometry. Lung mononuclear cells from naïve and latent mice were also stimulated ex vivo with LPS or ConA for 24hr at 37°C and supernatants were subjected to ELISA to measure IL-6 and TNFα cytokine levels.

Results: We report the following observations: (1) MCMV-infected mice showed a significant decrease in the average percentage of total CD3^+ T cells, CD3^+CD8^+ and CD3^+CD4^+T cells whereas the absolute number per gram of lung tissue of these cells was not notably different than uninfected mice; (2) A significant reduction in the B cell population and a significant increase in the NK cell population were detected (both average percentage and absolute number/gm of lung tissue) in latently infected mice compared to uninfected mice; (3) A significant increase in both the percentage and absolute number of Gr-1^+ myeloid cells, CD11b^+ cells, and cells resembling myeloid derived suppressor cells (CD11b^+Gr-1^+) was observed in lungs of latently infected mice; (4) Compared to cells from uninfected mice, lung cells from latently-infected mice were impaired for TNFα and IL-6 cytokine production after stimulation with either ConA or LPS.

Conclusions: Viral latency is an inevitable outcome of cytomegalovirus infection. During latency, infectious virus cannot be detected and no clinical symptoms are apparent. Nevertheless, our findings show that viral latency drastically alters the immunological environment of the lung. Specifically, the percentage of both T and B lymphocytes is diminished in the latent lung whereas the percentage of NK cells and myeloid cells is increased. Furthermore, ex vivo stimulation experiments suggests that latency impairs the responsiveness of lung cells to con A or LPS, possibly due to the increase in the percentage of myeloid-derived suppressor cells that accumulate in the latent lung. Further research will focus on how these immunological findings relate to CMV-related lung injury upon trauma or injury as well as the mechanism by which viral infection remodels the immunological environment of the lung.
Sonographic evaluation of intravascular volume status in the surgical intensive care unit: a prospective comparison of subclavian vein and inferior vena cava collapsibility index

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Introduction: Traditional methods for intravascular volume status assessment are invasive and are associated significant complications. While focused bedside sonography of the inferior vena cava (IVC) has been shown to be useful in estimating intravascular volume status, it may be technically difficult and limited by patient factors such as obesity, bowel gas, or postoperative surgical dressings. The goal of this investigation is to determine the feasibility of subclavian vein (SCV) collapsibility as an adjunct to IVC collapsibility in intravascular volume status assessment.

Methods: A prospective study was conducted on a convenience sample of surgical intensive care unit (SICU) patients to evaluate interchangeability of IVC collapsibility index (CI) and SCV-CI. After demographic and acuity of illness information was collected, all patients underwent serial, paired assessments of IVC-CI and SCV-CI using portable ultrasound device (M-Turbo™ by Sonosite, Bothell, WA). Vein collapsibility was calculated using the formula [Collapsibility (%) = (Max diameter - Min diameter)/Max diameter x 100%]. Paired measurements from each method were compared using Correlation Coefficient and Bland-Altman measurement bias analysis.

Results: Thirty-four patients (mean age 56 years, 38% female) underwent a total of 94 paired SCV-CI and IVC-CI sonographic measurements. Mean APACHE II score was 12. Paired SCV and IVC collapsibility indices showed acceptable correlation (R2=0.61, p<0.01) with acceptable overall measurement bias [Bland-Altman mean collapsibility difference (IVC-CI minus SCV-CI) of -3.2%]. In addition, time needed to acquire and measure venous diameters was shorter for the SCV-CI (70 sec) when compared to IVC-CI (99 sec, p<0.02).

Conclusions: Subclavian vein collapsibility assessment appears to be a reasonable adjunct to IVC-CI in the surgical ICU patient population. The correlation between the two techniques is acceptable and the overall measurement bias is low. In addition, SCVCI measurements took less time to acquire than IVC-CI measurements, although the clinical relevance of the measured time difference is unclear.
HB-EGF augments the ability of mesenchymal stem cells to attenuate intestinal injury

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Introduction: Mesenchymal stem cells (MSC) are pluripotent, self-renewing progenitor cells with proven therapeutic efficacy in multiple animal models of ischemic end-organ injury. We have previously shown that heparin-binding EGF-like growth factor (HB-EGF) promotes MSC proliferation and migration and decreases MSC apoptosis in vitro. We have also shown that HB-EGF and MSC have potent intestinal cytoprotective effects in animal models of intestinal ischemia/reperfusion (I/R) injury, with a synergistic effect when HB-EGF is combined with MSC therapy. The goal of the current study was to clarify whether this synergistic effect was due to a direct effect of HB-EGF on MSC.

Methods: Amniotic fluid-derived MSC were isolated from adult pan-EGFP mice, and pluripotency confirmed by induced differentiation. MSC were either transfected with an HB-EGF plasmid to produce HB-EGF-overexpressing MSC, transfected with a scrambled plasmid control (control-transfected-MSC), or were non-transfected (MSC). Mice were subjected to segmental terminal ileal ischemia for 60 minutes and randomized to: 1) no therapy; 2) HB-EGF intraluminally (IL); 3) MSC intraperitoneally (IP); 4) HB-EGF IL + MSC IP; 5) HB-EGF-transfected-MSC IP; or 6) control-transfected-MSC IP. After 24 hours of reperfusion, intestines were harvested, histologic injury graded from 1-5, MSC engraftment quantified, and intestinal permeability determined.

Results: Histologic injury grade was significantly decreased for all treatment modalities (HB-EGF 0.9 ± 0.3 p<0.01, MSC 1.1 ± 0.7 p<0.01, MSC+HB-EGF 1.0 ± 0.6 p<0.01, HB-EGF-transfected-MSC 0.8 ± 0.3 p<0.01, and control-transfected-MSC 1.0 ± 0.5 p<0.01) compared to injury alone (3.4 ± 0.6). MSC preferentially engrafted into injured terminal ileum compared to uninjured jejunum (1.1 ± 0.5 vs. 0.2 ± 0.2 MSC/villus). There was increased engraftment into injured ileum with combined MSC+HB-EGF (1.5 ± 0.4) or with HB-EGF-transfected-MSC (1.8 ± 0.3), compared to treatment with non-transfected MSC (1.1 ± 0.5) or control-transfected MSC (1.1 ± 0.2, all p<0.05). Intestinal permeability was significantly improved for all treatment groups (HB-EGF 30.3 ± 17.8 p<0.01, MSC 42.2 ± 15.2 p<0.01, MSC+HB-EGF 12.6 ± 7.6 p<0.01, HB-EGF-transfected-MSC 10.4 ± 7.1 p<0.01 and control-transfected-MSC 48.9 ± 13.5 p<0.01) compared to injury alone (92.9 ± 30.9). Intestinal permeability was most improved in the MSC+HB-EGF and the HB-EGF-transfected-MSC groups compared to the other groups (all with p<0.05).

Conclusion: These data demonstrate that delivery of HB-EGF-overexpressing MSC intraperitoneally protects the intestines from I/R injury to the same extent as treatment with non-transfected MSC delivered intraperitoneally in combination with HB-EGF delivered intraluminally. This suggests that HB-EGF interacts directly with MSC to augment MSC transplantation efficacy. Improved understanding of the synergistic therapeutic effects of HB-EGF and MSC may facilitate the development of novel therapeutic strategies for the management of intestinal injury.
Hyponatremia increases expression of apoptosis related proteins and infarct size

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Introduction: Hyponatremia (serum Na < 135mEq/L), is the most common electrolyte abnormality encountered in clinical practice. Hyponatremia adversely affects outcomes in a variety of clinical conditions. We have previously demonstrated that 21% of patients undergoing cardiac surgery have preoperative hyponatremia and it was associated with a 31% increase in the hazard of mortality after cardiac surgery. The mechanisms responsible for the adverse effects of hyponatremia on cardiac surgery patients are unknown. We postulated that the adverse effect of hyponatremia may be related to increase in reactive oxygen species production. We used an hyponatremic rat model to investigate the effects of hyponatremia on infarct size, cardiac function, reactive oxygen species production and apoptosis.

Methods:
1. Induction of hyponatremia in rats: Sprague-Dawley rats were divided into two groups: A) control group; B) hyponatremia group. Hyponatremia was induced in rats by the infusion of DDAVP with a subcutaneous mini-pump (5 ng/hr) and by feeding the rats with AIN-76 liquid diet for minimum of 14 days.
2. Myocardial infarction model: Myocardial infarction was induced by ligation of the left anterior descending coronary artery (LAD) for 24 hrs.
3. Echocardiography: 2D and M mode echocardiography were performed before and after LAD ligation in the parasternal short axis and LVEF was calculated by averaging 3 cardiac cycles.
4. Myocardial Infarct size measurement: The area at risk and infarct size were determined by TTC staining. The pictures and quantification of the area were measured using MetaMorph software.
5. TUNEL assay: TUNEL staining was performed with the In Situ Cell Death Detection Kit (Fluorescein). After staining, the slides were then examined by using a Zeiss LSM 510 confocal microscope. The intensity of the fluorescence was quantified by software Image J.
6. Western Blotting analysis: Proteins were isolated from the infarct and non-infarct rat heart tissues and separated through 4-12% SDS-PAGE. P53, Bcl-2, 3-Nitrotyrosine were detected by their corresponding primary antibodies, second antibodies and ECL detection reagent.
7. Confocal Microscopy: Myocardium was stained with anti-3-Nitrotyrosine primary antibody and Texas Red. Images were captured by confocal microscopy and intensity of fluorescence quantified by image J.

Results:
Infarct size was larger in hyponatremia rats than control rats (47 ± 2% vs. 38 ± 3% of area at risk, p<0.05), left ventricular ejection fraction was reduced by 16.5 ± 1.8% in control rats vs. 24.18 ± 1.3% in hyponatremia rats (p<0.05). Hyponatremia rat heart tissues have higher percent of apoptotic cardiomyocytes in both non-infarct area and infarct area as compared to that of the control (non-infarct: 75.5 ± 1.46 vs 60.69 ± 3.77, p<0.05; infarct: 96.04 ± 4.0 vs 77.53 ± 0.61, p<0.05). The p53 and 3-Nitrotyrosine protein expression are higher in hyponatremia group compared to the control group both in non-infarct tissue and infarct tissue. The expression of Bcl-2 protein is much lower in the hyponatremia group than that in the control group both in non-infarct tissue and infarct tissue.

Conclusions:
1. Hyponatremia increases infarct size compared to control.
2. The reduction of left ventricular ejection fraction is larger in the hyponatremia group than that in the control group.
3. Hyponatremia induces more myocyte apoptosis than control in infarct and non-infarct areas.
4. Hyponatremia increases the myocardial expression of the proapoptotic protein p53 and decreases the expression of the anti-apoptotic protein Bcl-2 both in infarct and non infarct areas.
5. Hyponatremia increases protein nitration as demonstrated by increased expression of 3-Nitrotyrosine by Western Blot and immunofluorescence. This is an indirect evidence of increased peroxynitrite production.

Our results suggest that hyponatremia impairs tolerance to ischemia by increasing post translational nitration of proteins and inducing apoptosis. Modulation of nitration and apoptosis may be a strategy to improve outcomes in hyponatremic patients.
Adaptations in gene & protein expressions within different regions of the left ventricle after an acute myocardial infarction

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Introduction: Myocardial infarction (MI) is the leading cause of death worldwide. After an acute MI, maladaptive global and regional myocardial gene expression takes place which can ultimately lead to heart failure. Using our unique model of ovine MI, we aim to study the regionality of myocardial gene and protein expression.

Methods: Platelets from adult sheep (n=5) were isolated to create an autologous thrombus. Under fluoroscopic guidance, cardiac catheterization was performed with embolization of the mid-left anterior descending artery to create an acute myocardial infarction. Regional myocardial tissue from the infarct zone, adjacent zone (defined as < 0.5 cm from the border of the infarct zone) and the remote zone from each ovine were collected on the third day. Histological and immunofluorescence staining was carried out in the different myocardial regions. Tissue harvested was probed for gene expression, using quantitative real-time PCR, as well as protein expression, using Western blot analysis/densitometry, in order to assess: 1) calcium-channel handling (SERC2A), 2) apoptosis (Bcl-2), and 3) cell survival (PI3K). An analysis of variance (ANOVA) was used to compare the 2^{ΔΔCt} values as well as the Western blot densitometric values among the different tissue samples.

Results: Expression levels were compared to control tissue (n = 5 sheep). The remote regions isolated from the embolized animals showed little variability from our control animals (p_{Bcl-2}=0.9696, p_{PI3K}=0.9757, p_{SERC2A}=0.4932). Relative to control tissues, the border zone showed a 4-fold increase in expression of Bcl-2 (p=0.0052) and a 1.5-fold decrease in expression of SERC2A (p=0.0188). The infarct region showed a 7-fold increase in expression of Bcl-2 (p < 0.0001) and a 32-fold decrease in expression of SERC2A (p < 0.0001). PI3K results did not show any significant fold changes in expression among the different regions compared to control tissue (p_{border}=0.9850, p_{infarct}=0.3056). This was correlated with Western blots/densitometry (not shown).

Conclusions: Early regional differences in gene expression exist in the early period after an acute myocardial infarction. The manipulation of these early genetic changes could lead to potential therapeutic targets to mitigate the effects of up-regulation and hence, prevent ischemic heart failure.
Introduction: Traumatic brain injury (TBI) is associated with immediate inflammatory-associate complications, including edema, that contribute to impaired functional recovery. Moreover, mental health deficits including depression often develop and persist in the years following TBI. As the majority of individuals who suffer a TBI are juveniles and young adults, the increased risk of long-term neurological and depressive complications is a significant concern. Unfortunately, TBI-associated depression is often resistant to anti-depressant treatment and there are no effective pro-active treatment strategies for immediate TBI-induced complications. We have preliminary evidence that immediate inflammatory events elicited by TBI promote a more inflammatory profile (priming) of the CNS resident immune cells, microglia, leading to increased prevalence of depressive behavior. Methylene blue (MB) is an agent used clinically to reduce inflammation associated with septic shock and ischemia. We hypothesized that early intervention with MB would ameliorate the immediate inflammatory events associated with TBI, improve functional recovery, and reduce depressive-like behavior corresponding to reduced microglia priming.

Methods: Adult (3 mo) male BALB/c mice were given a sham injury or a midline fluid percussion injury to elicit a moderate and diffuse TBI. This model of TBI closely models mild to moderate human concussions in that it causes a brief period of unconsciousness and acute cognitive and behavioral deficits, but does not induce gross neuronal death, tissue cavitation, or cause long-lasting changes in motor function. Within 15 min of injury mice were injected intravenously (i.v.) through the tail vein with vehicle or 2 mg/kg MB. After 24 h brains were collected to measure edema and immediate changes in brain inflammation. In another subset of mice, motor coordination, nesting behavior, and depressive-like behavior were evaluated up to one week after injury. Functional recovery was assessed by the ability to engage in normal self-care behaviors (nest building) and proper motor coordination (rotarod apparatus). Depressive-like behavior was measured as the amount of time spent immobile in the tail suspension test, with increased immobility serving as a marker for increased depressive-like behavior. Mice were then left undisturbed until one month after injury when brains were collected for analysis of microglia priming (i.e., increased major histocompatibility complex [MHC]II) by flow cytometry and qPCR.

Results: Preliminary data show that early intervention with MB successfully ameliorated TBI-induced edema and brain inflammation. In particular, inflammatory IL-1â expression was reduced whereas anti-inflammatory IL-4 expression was increased. Moreover, the percentage of inflammatory peripheral immune cells that trafficked to the brain was reduced with MB treatment. Although nest building was not affected by MB treatment, MB treatment improved functional recovery as assessed by motor coordination, and ameliorated depressive-like behavior. These functional and behavioral improvements corresponded with a reduction in the percentage of primed and MHCII+ microglia one month after injury.

Conclusions: Together these results indicate that early intervention with MB after moderate TBI reduces both immediate and long-lasting consequences of TBI. Thus, MB treatment could reduce immediate life-threatening complications, including edema, corresponding to a reduction in morbidity and mortality after TBI. Moreover, early MB treatment has the potential to promote long-lasting neurological improvements in TBI patients including reduced prevalence of depression. Thus, MB infusion after TBI may be a new strategy for treatment of mild to moderate TBI.
MicroRNA-1 induction impairs ischemic wound healing attenuating keratinocyte migration and angiogenesis


Introduction: Ischemia is a key factor that limits dermal wound healing. We have developed a murine model to identify the molecular mechanisms of gene regulation that result in impaired healing in ischemic wounds. MicroRNAs (miR) play an important role in regulating gene expression in ischemic wounds and in this study we have identified miR-1 as a key culprit in impairing wound healing.

Methods: Recent studies show that aquaporin 3 (AQP3) supports keratinocyte migration. AQP3 knockdown compromised human keratinocyte cell migration (n=4, p<0.05). We observed that AQP3 mRNA and protein expression is induced in non-ischemic wounds while this induction is significantly blunted in ischemic wounds (n=4, p<0.05). To study AQP3 gene regulation by miRs, microRNA profiling was done comparing skin, non-ischemic and ischemic wound samples. Among the differentially regulated microRNAs, miR-1 was significantly upregulated and the result was validated using QPCR (n=3, p<0.05). miR-1 induction, using miR-1 mimic, retarded keratinocyte migration as determined by scratch assay (n=4, p<0.05). We identified c-met, a regulator of AQP3, as a direct target of miR-1 in keratinocytes (n=3, p<0.05) by a 3' UTR-luc assay. c-met knockdown in keratinocytes downregulated AQP3 protein expression and impaired cell migration (n=4, p<0.05). AQP3 also blunted H2O2 uptake by cells which is known to be a required signaling messenger for healing. Additionally, miR-1 was found to inhibit tube formation in HMEC cells and endothelial cell migration. miR-1 is predicted to directly silence VEGF-A and thus impair angiogenesis, another critical requirement for successful wound healing.

Results:
• miR-1 is induced in ischemic wounds.
• miR-1 silences c-met in keratinocytes which is required for AQP3 induction and keratinocyte migration.
• Reduced AQP3 blunted H2O2 uptake which is required for cell signaling.
• miR-1 also impaired endothelial cell migration and angiogenic tube formation.

Conclusions: In conclusion, we have identified miR-1 as a key therapeutic target for management of chronic wound healing. We propose that strategies to inhibit miR-1 in ischemic wounds will be productive to restore healing of ischemic wounds.
Near infrared fluorescent cholangiography (NIRF-C) for biliary imaging during laparoscopic cholecystectomy

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Introduction: Laparoscopic cholecystectomy is one of the most commonly performed surgical procedures in the United States. The most feared complication and major source of morbidity remains bile duct injury (BDI). BDI is an important target to improve outcomes for one of our most common operations. Radiographic intra-operative cholangiography (IOC) is the gold standard for bile duct imaging in the operating room, but invasiveness and time requirements limit its use. Near infrared fluorescence cholangiography (NIRF-C) is a recent imaging modality that utilizes a fluorescent dye, indocyanine green (ICG), which is injected intravenously and excreted in the bile. A laser is used to excite the agent and the biliary tree can be seen through the surrounding soft tissue with an image filter on the laparoscope. We aim to describe the effectiveness of NIRF-C in identifying pertinent biliary anatomy during laparoscopic cholecystectomy

Methods: Patients underwent laparoscopic cholecystectomy with NIRF-C after intravenous injection of 2.5 mg of ICG approximately one hour prior to their operation. The ability of NIRF-C to identify pertinent biliary anatomy was documented and recorded as an incidence. When all data regarding NIRF-C had been collected, patients underwent intra-operative cholangiography for comparison.

Results: Eighteen patients underwent NIRF-C and attempted IOC in our series. The mean BMI was 29.4 for all participants. The patients underwent cholecystectomy for biliary colic (n=10), chronic cholecystitis (n=5), biliary dyskinesia (n=2), or gallstone pancreatitis (n=1). No complications occurred in our series. The incidence of biliary structures identified before dissection and after complete dissection was as follows: common hepatic duct (50% and 78%), hepatic duct/cystic duct junction (39% and 83%), cystic duct (78% and 94%), common bile duct (56% and 72%) IOC was successfully completed 89% of the time. Failure of IOC was due either to technical difficulty or lack of fluoroscopic availability. There was no lack of congruence between NIRF-C and IOC. No anatomic varients were identified in our population.

Conclusions: After dissection, NIRF-C’s ability to identify the cystic duct, its junction with the common hepatic duct, and the common bile duct approached that of IOC when IOC failures are included. It also provides a guide to initial dissection. NIRF-C represents a quick, safe, and non-invasive alternative to IOC for real time intraoperative biliary imaging that holds great promise in the proper population.
The role of microRNA-155 in a murine model of non-alcoholic steatohepatitis

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Introduction: Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide with increasing incidence and mortality. Risk factors include acquisition of the Hepatitis B or C virus, alcohol use, and non-alcoholic steatohepatitis (NASH). With the increase in obesity in the developed world over the last few decades, which is itself a risk factor for NASH, the incidence of NASH and NASH-associated HCC is on the rise. Currently, there is no effective therapy for NASH, allowing for progression through its natural history from inflammation to fibrosis, cirrhosis, and ultimately, hepatocellular carcinoma. The lack of treatment for NASH necessitates understanding the mechanism of its pathogenesis. To this end, our lab has used a murine model of HCC in which all mice fed choline-deficient and amino acid defined (CDAA) diet develop NASH prior to the development of HCC with stepwise hepatic pathological changes that mirror the progression of steatohepatitis to HCC in humans. We have noted a consistent upregulation of miR-155, a proinflammatory microRNA, from an early stage of feeding the CDAA diet which correlates with development of NASH. Importantly, increased miR-155 level in humans is an independent predictor of poor prognosis and survival and also correlates with invasion in HCC patients. The involvement of microRNAs in malignancies has become an increasingly important aspect in the field of oncology due to the potential for novel therapeutics directed at these small non-coding RNAs that can post-transcriptionally regulate a number of genes and play a role in oncogenesis. We hypothesize that upregulation of miR-155 plays a causal role in NASH and HCC and that the absence of miR-155 will result in decreased inflammation, fibrosis, and eventually, development of HCC. The overall goal of this study is to establish the role of miR-155 in the pathogenesis of HCC for future development of anti-miR therapy.

Methods: Male C57BL6 mice (control) and miR-155KO mice were fed the CHOW diet (control) and the CDAA diet for a duration of 8 weeks. Blood and organs were then harvested for pathologic and serologic studies and protein and RNA extraction. Additionally, body and liver weight, histology, serology, and presence or absence of steatosis were also noted. From the hepatic tissues, gene expression at the protein and mRNA levels were quantified using Western gel electrophoresis and RT-PCR.

Results: miR-155KO mice exhibited increased liver to body weight ratio as compared to control mice in the absence of increased body weight and H&E staining of these samples revealed increased steatosis in the KO mice. Additionally, reduced inflammation was seen in the KO mice and correlated with decreased NF-kappaB activity. NF-kappaB is a well known regulator of inflammation. Reduced expression of the p65 subunit of NF-kappaB was seen at both the mRNA and protein level. TRAF-3, a negative regulator of NF-kappaB, was identified as a marker of miR-155 at both the mRNA and protein level, and was verified using a UTR reporter assay suggesting that TRAF-3 may be a novel target of miR-155.

Conclusions: Increased liver weight and increased steatosis suggests that miR-155 plays a role in hepatic lipid metabolism. Reduced inflammation in the KO mice along with reduced expression of the p65 subunit of NF-kappaB suggests that reduced expression of p65 may contribute to decreased NF-kappaB activity as the mechanism of miR-155 inflammation. Additionally, TRAF-3 which is a known negative regulator of NF-kappaB, has been identified as a novel target of miR-155.
Keratinocyte-directed conditional ablation of dicer impairs wound healing via induction of P21\(^{\text{WAF1/CIP1}}\)

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Introduction: Homeostasis of adult tissue is maintained by molecular silencers called microRNA (miRs) that post-transcriptionally silence coding genes. Injury transiently silences these silencers to unleash adult tissue development towards the healing process. Once the wound is closed, miRNA biogenesis is bolstered to turn off tissue development averting neoplasia. We report that Dicer, one of the key RNase III responsible for miRNAs maturation, plays an important role in re-establishing miR-dependent silencing at the time of wound closure. Dicer expression is dysregulated in several human disease conditions. Compromised dicer function predicts poor health outcomes.

Methods: Keratinocyte-specific conditional (K14/Lox-Cre) dicer ablated mice were generated. Excisional wounds were developed on the dorsal skin.

Results: We observed that non-healing diabetic wounds feature compromised dicer expression. miRNA expression profiling of skin and wound-edge tissue revealed a global up regulation of miRNAs during wound closure on day 14 post wounding. During wound closure, dicer protein expression increased by >2.5 fold (n=4; p<0.001). Barrier function of the skin was compromised in keratinocyte-specific dicer ablated mice because of impaired loricrin expression. In vitro studies with HaCaT human keratinocytes showed that loricrin expression was inversely related to the expression of the cyclin dependent kinase inhibitor p21\(^{\text{WAF1/CIP1}}\). Real time PCR of p21\(^{\text{WAF1/CIP1}}\) from laser captured wound-edge keratinocytes revealed more than 2.5 fold elevated mRNA expression in dicer ablated skin epidermis compared to normal epidermis (n=6; p<0.001). Increased expression of p21\(^{\text{WAF1/CIP1}}\) in keratinocyte-specific dicer ablated wound edge tissue was also confirmed by Western blot and immunohistochemistry. Suppressing p21\(^{\text{WAF1/CIP1}}\) by p21\(^{\text{WAF1/CIP1}}\) anti-sense adenovirus in keratinocyte-specific conditional dicer ablated mice improved wound healing suggesting a role of dicer in the suppression of p21\(^{\text{WAF1/CIP1}}\).

Conclusions: These results establish that dicer enables p21\(^{\text{WAF1/CIP1}}\) silencing helping re-establish barrier function of the wounded skin.